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A new auxin-like active substance from chestnut crown gall

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Preface

It is well known that fresh buds of chestnut tree (*Castanea crenata* SIEB. et ZUCC.) infested by larvae of gall wasps (*Dryocosmus kuriphilus*, Cynipidae) abnormally grow to form galls, so called chestnut crown gall, in size from 1 to 3 cm. With a view to clarifing an inducing fctor of hypertrophy of the buds, We examined some of biological activities of methanol extracts of fresh chestnut crown galls and larvae of gall wasps, and healthy buds of the chestnut tree. The rice lamina joint[1] and the avena straight growth test for auxin activity, and the cucumber cotyledon assay[2] for gibberellin and kinetin activity were carried out in our laboratory. A strong response of the methanol extract of fresh chestnut crown galls to the rice lamina joint test was only observed, but the other methanol extracts did not show a valuable response. Here we wish to report the partial purification and some biological activities of the auxin-like active substance in fresh chestnut crown galls.

Materials and Methods

Fresh chestnut crown galls (3.5 kg) collected at the suburbs of Tsuruoka city in mid-June, 1970, were crushed and immersed in methanol for a month at room temperature. The mixture was filtered by suction, and the residue was again immersed in methanol for a week. The methanol extracts were combined, evaporated in vacuo and extracted with ethyl acetate. The ethyl acetate soluble portions were fractionated into a neutral, a basic and an acidic fraction. Only the neutral fraction showed a strong activity in the rice lamina joint test. The neutral fraction was chromatographed on a silica gel column, and the active fraction was eluted with ethanol-chloroform (8 : 92). Further the active fraction was subjected to successive silica gel column chromatography with the mixture of ethyl acetate-benzene (9 : 1) to give a crude active substance (17.9 mg). The final purification was carried out by a preparative thin layer chromatography (Kieselgel G, ethyl acetate ; R_f value : 0.25), then the active colorless substance (substance I, 3.2 mg) was obtained.

Results and Discussion

Substance I was detectable as a purple spot under ultraviolet light after spraying with sulfuric acid containing 0.5 % vanillin, or a light brown one over vapor of iodine on a TLC plate. It gave negative EHRLICH and SALKOWSKY reactions at the amount of 5 μg on a TLC plate. The absorption coefficients in methanol solution in the UVspectrum ($E^{1\%}_{1\,cm}$ 616 at 204 nmand $E^{1\%}_{1\,cm}$ 77 at 279 nm) are much smaller than those of indolyl compounds[3]. These chemical and physical data suggest that substance I is not an indole compound. This was supported from its NMR spectrum : there was no signal in the aromatic proton region (δ6.5~9.0). The infrared spectrum of chloroform solution showed charactaristic strong absorptions at 3500~3200 (OH), 1715 (C=O) and 1605 (C=C) cm^{-1} (Fig. 1).

The test solution of substance I (0.01 ppm) showed the same activity as that of IAA solution (1.0 ppm) in the rice lamina joint test. In the avena straight growth test, the activity nealy corresponded to that of IAA.

The auxin activity of substance I focused at R_f 0.9~1.0 on the paperchro matogram (No 50 Toyo-Roshi, n-butanol-ammonia-water, 4 : 1 : 1). This activity could not be detected at the same position on the paper chromatogram of the methanol extract of healthy buds. but a slight response to the rice lamina joint test was observed at R_f 0.3~0.5, corresponding to IAA and chlorinated 3-indolylacetic acid[4].

Although ethyl acetate-soluble neutral fractions of the methanol extracts

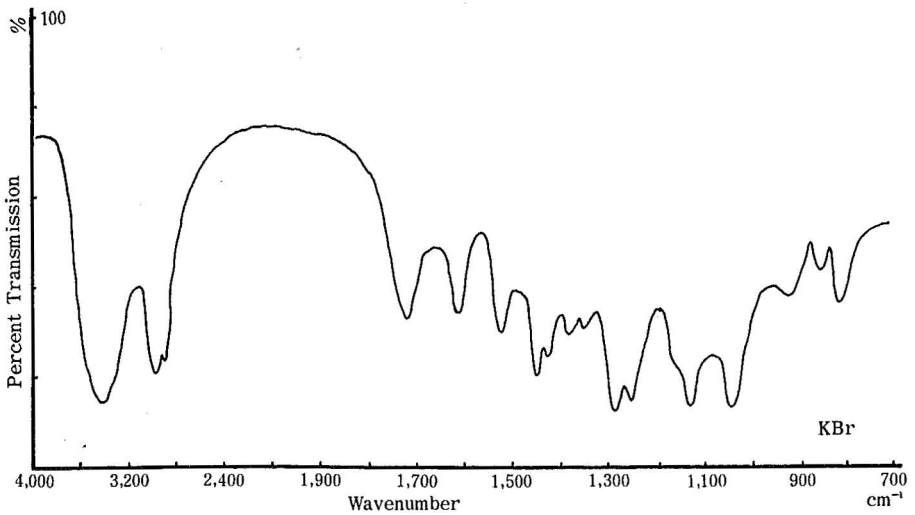

Fig. 1. Infrared absorption spectrum of substance I

obtained from healthy buds (150g) of chestnut trees and larvae of the wasps in galls (100g) were worked up in a similar manner as in the case of the galls extract, their activities in the rice lamina joint test could not be detected even at the final stage.

It is clear that chestnut crown gall itself includes a substance having much higer activity than that of IAA in the rice lamina joint test. Several plant growth regulators such as IAA, methyl indolacetate, indolacetamide, indolacetaldehyde dimethylacetal[5] and jasmonic acid[6] have been isolated from the gall tissue. On the basis of spectral data and chemical properties, substance I apparently differs from these substances. This finding is of interest in connection with the chemical elucidation of formation mechanism of chestnut crown gall. On the other hand, there are several reports on non-indolic auxins which are active in various auxin tests. Recently, MUNAKATA et al[7] reported the isolation of a non-indolic auxin. named corn factor, from corn oil whose activity was about 2500 times that of IAA in the rice lamina joint test.

From the structural point of view also we are so interested in these non-indolic auxins.

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Literature Cited

1) MAEDA, E., Physiol. Plant., **18**, 813 (1965).
2) KNYPL, J. S., Nature, **224**, 1025 (1969).
3) JERCHEL, D., and STAAB-MULLER, R., Z. Naturforsch., **9b**, 411 (1954).
4) MARUMO, S., Chem. Regulation of plant, **4**, 143 (1969).
5) YOKOTA, T., OKABAYASHI, N., TAKAHASHI, N., SHIMURA, I., and UMEYA, K., Plant Growth Substance 1973, Hirokawa publishing co., Tokyo (1974), p28.
6) TAKAGI, H., OKABAYASHI, N., YOKOTA, T., TAKAHASHI, N., SHIMURA, I., and UMEYA, K., Abstracts of papers, Annual Meeting of Agricultural Chemical Society, Sapporo, Japane, July, 1975, p349.
7) MUNAKATA, K., KATO, N., and IKEDA, M., Plant Growth Substance 1973, Hirokawa publishing co., Tokyo (1974), p39.

Summary

1) *Dryocosmus kuriphilus* a cecidogenetic wasp of chestnut tree (*Castanea crenata*), causes suppression of a normal sprout growth to form a gall on the stem. In ordor to find aplant growth substance in the chestnut crown gall we have xamined.

2) It was found that the gall contains an auxin-like active substance. The active principle, named substance I, was isolated as colorless semisolid using a wide variety of chromatographical technique.

3) Auxin activity of substance I showed about 100 times that of indolacetic acid in the rice lamina joint test. Substance I showed no signal in the indolyl proton region in the NMR spectrum and gave negative EHRLICH and SALKOWSKY reactions. These facts suggest it is not an indole compound.

4) In connection with the chemical elucidation of formation mechanism of chestnut crown gall and the longstanding question of the existence of non-indolic auxin in plant, substance I is of interest.

要　　旨

1) クリの木 *Castanea conata* につくクリタマバチ *Qryocosmus kuriphilus* は，芽の正常な発育を阻害し，枝に虫瘿を形成する．我々は，芽の肥大を引きおこす原因となる植物生長物質を見い出すために，実験を進めた．

2) 虫瘿には，ラミナジョイントテストによって，強いオーキシン活性物質が含まれている事を見い出した．Substance I と名づけられたこの物質は，色々なクロマトグラフを用いて無色・半固体状のものとして単離された．

3) Substence I のオーキシン活性は，ラミナジョイント試験で，インドール酢酸の100倍であった．Substance I の NMR スペクトルは，インドール核の水素原子である事を示す領域に，シグナルがな，くインドール核の存在を検出する EHRLICH 試薬および SALKOWSKY 試薬に陰性であり，Substance I はインドール化合物でないと考えられた．

4) クリタマバチ虫瘿形成のメカニズムを化学的に解明することや，植物中に非インドール系オーキシンが存在するかどうかという古くからの疑問に関連し，Substance I は，興味深い．